VWF Exon 28 Sequence Analysis

Versiti offers DNA sequencing of *VWF* exon 28 (order code 1284) for detection of germline variants associated with type 2B or type 2M von Willebrand disease (VWD).

Von Willebrand disease (VWD) is a common inherited bleeding disorder with a reported incidence ranging from 0.01% to 1%. VWD is classified into subtypes of quantitative (types 1 and 3) and qualitative (type 2) defects, caused by pathogenic variants in *VWF*. Type 1 VWD, characterized by partial deficiency of von Willebrand factor (VWF), is inherited as an autosomal dominant disorder with variable penetrance. Type 1C VWD is a variant of autosomal dominant type 1 VWD characterized by decreased survival (increased clearance). The defects observed in type 2 VWD include defects in formation of multimers (types 2A), increased susceptibility of VWF to degradation by proteases (type 2A), defects in platelet binding with intact multimers (type 2M), enhanced interaction of VWF with platelets (types 2B, and *GP1BA*-related platelet-type VWD), decreased interaction with factor VIII (type 2N), and decreased interaction with collagen (a rare form of type 2M). Types 2B, type 2M, and the majority of type 2A cases have an autosomal dominant inheritance pattern, while type 2N is an autosomal recessive disorder. Type 3 VWD is characterized by severe quantitative deficiency with a virtual absence of VWF and is inherited as an autosomal recessive disorder. Platelet-type VWD is caused by pathogenic gain-of-function variants in platelet glycoprotein 1b encoded by the *GP1BA* gene, and will not be detected by *VWF* analysis.

Genetic testing of *VWF* exon 28 offers the most clinical utility for the diagnosis of types 2B and 2M VWD, in confirming the VWD type to facilitate selection of appropriate medical therapy, and accurately determine recurrence risks. Laboratory findings suggestive of type 2M VWD include evidence of abnormal interaction of platelets with VWF (as suggested by a low ratio of VWF ristocetin cofactor or GP1bM activity to VWF antigen level) in the setting of a normal VWF multimer distribution. Alternatively, the additional laboratory findings suggestive of type 2B VWD may include absence of high molecular weight multimers, abnormal low-dose ristocetin-induced platelet aggregation result, thrombocytopenia or an abnormal platelet-VWF binding assay.

If other types of VWD are clinically suspected, alternative genetic testing strategies may be considered. *VWF* genetic analysis (order code 4855) is recommended for types 1 and 3 VWD as well as for other types, including 1C and 2A; although these latter types have been associated with variants in certain exons of *VWF*, evolving knowledge has revealed that pathogenic variants causing these phenotypes occur across the gene, and therefore testing of limited exons is no longer a recommended approach. Variants causing type 2N VWD are located in specific factor VIII-binding functional domains in exons 17-21 and 24-27; for patients with low factor VIII and suspicion of type 2N VWD or patients with functional binding assays consistent with this diagnosis, VWD Type 2N Sequence Analysis (order code 1288) is available. In families with a specific VWD diagnosis in whom prior testing has identified a pathogenic variant that fully explains the phenotype, Targeted Familial Variant Analysis (order code 4970) is appropriate for evaluation of at-risk relatives or for prenatal diagnosis.

Indications for testing:

Diagnosis of von Willebrand disease, particularly when types 2B and 2M or the ristocetin-binding polymorphism D1472H are suspected

- Evaluation of abnormal VWF activity to antigen ratio, in the context of normal multimers or enhanced VWF-platelet binding
- Facilitate selection of appropriate medical therapy
- Identification of pathogenic variant(s) to allow for familial testing or prenatal diagnosis

For clinical questions about laboratory tests and test utilization support, contact Versiti Client Services (414) 937-6396 or 800-245-3117 (ext. 6250) to be directed to genetic counselors or clinical support team.

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Test method:

This next-generation sequencing assay analyzes the complete coding region of VWF exon 28 plus a minimum of 30bp of non-coding DNA, including intron-exon boundaries, and is compared to the build GRCh37.p13 reference sequence (VWF, NM_000552.3). These regions are captured by hybridization, amplified and sequenced by massively parallel sequencing. Regions will have a minimum coverage of 50x and those regions with less than 50 sequencing reads or low quality are supplemented with Sanger sequencing. All regions are covered by bidirectional analysis. Variants are identified by a customized bioinformatics pipeline, analyzed and comprehensively interpreted by our team of practicing hematologists with expertise in non-malignant hematology and laboratory diagnostics, scientists, and genetic counselors. All reported variants, including pathogenic, likely pathogenic, and variants of uncertain significance, are confirmed by Sanger sequencing. For prenatal testing, analysis of variable number tandem repeats (VNTR) is used to confirm results are not affected by maternal cell contamination.

Assay sensitivity and limitations:

The analytical sensitivity of this test is >99% for single nucleotide changes and insertions and deletions of less than 20 bp. NGS analysis is not designed to detect large deletions or duplications (>20 bp), or variants that are outside the regions sequenced. Low level mosaicism will not be detected by this sequencing methodology.

Clinical sensitivity

The clinical sensitivity for detecting pathogenic variants in individuals with a clinical diagnosis of types 2B and 2M VWD is approximately 99%. Although pathogenic variants in exon 28 have been associated with a majority of the cases of type 2A VWD, pathogenic variants associated with this type have also been identified in other areas of the VWF gene, such that testing of all exons via VWF Genetic Analysis (order code 4855) rather than testing of limited exons is suggested. Only a minority of pathogenic variants causing type 1 and 3 VWD are identified in exon 28. Exon 28 Sequence Analysis will distinguish type 2B VWD from platelet-type VWD, but sequence analysis of GP1BA is required to genetically confirm a diagnosis of platelet-type VWD.

Reporting of results:

Results are classified and reported in accordance with ACMG next-generation sequencing standards. Sequence variants predicted to be pathogenic, likely pathogenic, and of uncertain significance will be reported; variants classified as likely benign or benign are typically not reported but such data are available upon request.

Sequence variants are described using standard Human Genome Variation Society (HGVS) nomenclature (http://hgvs.org).

Specimen requirements:

Parental/Patient/Pediatric: 3-5 mL Whole blood (EDTA tube, lavender top), 2-5 mL Bone marrow (EDTA tube, lavender top), 3-4 Buccal swabs, or ≥1ug of DNA at ≥50ng/uL of High Quality DNA.

Fetal: 7-15 mL amniotic fluid, 5-10 mg chorionic villi; back up culture of amniocytes or chorionic villi is highly recommended. Cultured: Two T25 flasks cultured amniocytes or chorionic villi ($2x10^6$ minimum). Maternal blood sample of 3-5 mL Whole blood (EDTA tube, lavender top) is requested for all prenatal samples for maternal cell contamination studies. For questions please contact the laboratory to discuss sample requirements.

SHIP

Shipping requirements:

Ship on an ice pack or at room temperature. Protect from freezing. Place the specimen and the requisition into plastic bags and seal. Insert into a Styrofoam container; place into a sturdy cardboard box, and tape securely. Ship the package in compliance with your overnight carrier guidelines.

Send to:

Versiti Client Services
Diagnostic Laboratories
638 N. 18th Street
Milwaukee, WI 53233
800-245-3117, ext. 6250

ORDER

Required forms:

Please complete all pages of the requisition form. Clinical history (including patient's ethnicity, clinical diagnosis, family history and relevant laboratory findings) is necessary for optimal interpretation of genetic test results and recommendations. Clinical and laboratory history can either be recorded on the requisition form or clinical and laboratory reports can be submitted with the sample.

CPT Codes/Billing/Turnaround time:

Order code: 1284

For suggested CPT Codes, visit the Versiti.org/test menu

Turnaround time: 21 days

The CPT codes provided are subject to change as more information becomes available. CPT codes are provided only as guidance to assist clients with billing.

For additional information related to shipping, billing or pricing, please contact, Versiti Client Services (414) 937-6396 or 800-245-3117 (ext. 6250), or Labinfo@versiti.org

References

von Willebrand disease exon 28 references

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Variant interpretation references

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